FGC

extension note 02

Biotechnology: Potential Applications in Tree Improvement

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FOREST RENEWAL BC

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About the Forest Genetics Council of British Columbia

The Forest Genetics Council (FGC) of British Columbia is appointed by BC’s chief forester to guide tree improvement activities in the province. The Council’s Technical Advisory Committees (TACs) provide important avenues for technical communications among the different agencies that practise tree improvement activities in B.C., and coordinate business planning for each species in the provincial breeding programs. Council and its TACs include representatives from the forest industry, Ministry of Forests, Canadian Forest Service, universities, and Forest Renewal BC.

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1.0 Introduction

This note describes types of biotechnology that are being used or have potential applications in tree breeding and production of planting stock. It describes genetic engineering (GE) and the fundamental differences between GE and traditional selective breeding. It notes some of the concerns about the use of GE in tree improvement, and how British Columbia is responding to these issues.

The term "improvement" refers to the extent to which seed, seedlings, or trees exhibit a higher level of some desired trait than would wild-stand material.

2.0 Tree Improvement in British Columbia

In British Columbia, some 170 000 hectares of Crown forest land is harvested annually under a range of forest tenure agreements. By law, all such lands must be reforested to specified standards within a specified time period (often within three years of harvesting). Approximately 27% of the area harvested is reforested naturally, and 73% is regenerated through tree planting (B.C. Ministry of Forests 2000).

Since the mid-1960s, British Columbia has invested in the "improvement" of planting stock for some areas and species. Research has shown that many traits—including growth rate, size, form, timing of growth, seed germination, wood properties, leaf characteristics, cone morphology, pest resistance, and capacity to withstand climatic stresses—vary from one tree to another, and that these variations are in part due to genetic differences (Figure 1).

Figure 1
Variation in lodgepole pine growth attributed to genetic differences.

These lodgepole pine trees are all the same age and were grown on the same site. From left, seed was from low-, mid-, and high-elevation sources in the same valley. Such genetic differences are studied and seed planning zones are established to allow appropriate selection of seed sources for reforestation. Photo: M. Carlson.

The provincial tree improvement program takes advantage of these natural differences between individual trees of the same species. Tree breeding concentrates on selecting for growth rate and disease resistance without loss in wood quality in 10 commercial tree species. To date, the program has achieved genetic gains[1] in some species of up to 25% in wood volume production, with trees becoming large enough to harvest earlier than those from wild seed.

A few of the 200 million seedlings requested for sowing in 2000.

Of the approximately 200 million seedlings requested for sowing in 2000, 62% were produced from seed obtained from wild stands and some 37% from select sources (34% from seed orchards[2], 3% from superior provenances[3]).[4] A small proportion of trees are grown from rooted cuttings, or other asexually reproduced material.[5]

Tree improvement in British Columbia is one part of a larger program of forest gene resource management. The program also includes gene conservation initiatives, and guidelines to ensure that all planting stock—from orchard or wild-stand seed—is genetically diverse and adapted to the planting site.

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[1] Genetic gain is an estimate of the percentage increase in performance of an improved seedlot over that expected from wild-stand material. Gains are referenced to specific traits such as stem volume, wood density, or pest resistance.

[2] An orchard consisting of clones or seedlings from selected trees, isolated to prevent or reduce pollination from outside sources, and cultured for early and abundant production of seeds for reforestation. Material from seed orchards is referred to as genetic Class A.

[3] Provenances (seed sources) derived from natural stands that have been identified as having superior traits (e.g., growth performance) over that of local natural stand seed sources as shown through provenance trials. Referred to as genetic class B+.

[4] B.C. Ministry of Forests, Seed Planning and Registry system (SPAR).

[5] Most vegetative material used in B.C.'s reforestation program is from hedges and stoolbeds for species with a limited supply of high quality seed (e.g., yellow cedar) or for which vegetative propagation is preferred (e.g., hybrid and native poplars).

3.0 Tree Improvement Cycle

Tree improvement is a continual process of selection, testing, and breeding to increase the extent to which each generation of improved seedlings exhibits desirable traits—the "genetic gain" (Figure 2).

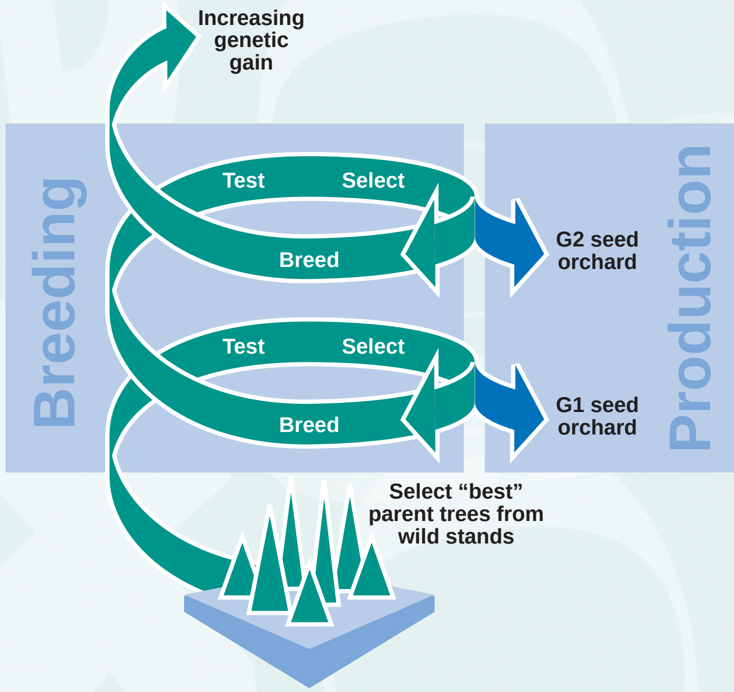

Figure 2
Tree breeding and seed production processes.

3.1 Selection

The first step in tree breeding is to find wild trees that exhibit desired characteristics (e.g., pest resistance, superior growth or form), to use as "parent trees." Scions (twig cuttings) are collected from these wild parents and grafted in orchards and breeding arboreta. Typically, hundreds—and sometimes thousands—of parent trees are selected across a broad forest land base to enable screening of many parents and to guarantee sufficient genetic diversity in future offspring.

3.2 Testing

In the first cycle of tree breeding, researchers try to identify those parent trees that carry the best genes for the desired traits. Some 30–40 seedling offspring ("progeny") from each wild parent tree are planted on each of several field sites (usually 3–10) representing the range of conditions within each of the seed planning zones[6] in which that species grows. These progeny tests measure seedling performance with respect to the desired trait (e.g., growth, stem form, pest resistance) over 10–20 years.

The parent trees whose offspring perform best over the variety of sites are assigned higher breeding values—the ability to pass on desirable traits to their offspring. Seed orchard managers use this information to establish the trees with the higher breeding values in orchards for future seed production.

[6] Seed planning zones are based on geographic patterns of genetic diversity as determined by long-term field trials called provenance tests.

3.3 Breeding

Researchers also implement a selective breeding program using the better of the wild tested parents and/or the better offspring of the original superior wild parents.

The goal of tree breeding is to increase the degree to which specific desired traits are expressed in each successive generation.

The tree breeder mates trees from this population according to a planned design and controls pollination to ensure that only the desired male pollens fertilize the female cones. The offspring from breeding programs provide further information about the breeding value of the parent trees and their families, and a new generation for future selection and testing.

The goal of tree breeding is to increase the degree to which the desired traits are expressed in each successive generation (Figure 3). In this way, selective breeding takes advantage of the natural variation found in wild stands (Figure 1).

Breeding programs for several species, including Douglas-fir and western hemlock on the Coast, and lodgepole pine and white spruce in the Interior, are entering a second cycle of breeding and selection.

Figure 3
Gains in growth rate from selective breeding in coastal Douglas-fir. These trees were grown on a single site at the Ministry of Forests Cowichan Lake Research Station. Photo: J. Woods.

3.4 Production

A vital output of tree breeding is improved reforestation materials grown from seed produced in seed orchards. In these orchards, grafted trees[7] from parents with the higher breeding values are planted in a random pattern and allowed to cross-pollinate. The "select seed"[8] they produce is sent to forest nurseries, which raise the large numbers of seedlings required for reforestation.

British Columbia's tree improvement and reforestation programs are designed to maintain genetic diversity across the forest land base. In seed orchards, genetic diversity is maintained and even enhanced by including many unrelated parent trees from a particular geographic area and allowing them to inter-mate.

Forest nurseries raise the large number of seedlings required for reforestation. Photo: D. Summers.

Technical standards for registration must be met before seed qualifies as "select." These include standards for genetic diversity, genetic worth (measures of genetic quality), adaptability to planting site (seed transfer guidelines), and field testing.

By law, orchards producing seed for Crown land reforestation must be licensed, and all seedlots[9] used on Crown land must be registered. The law also directs foresters to use seed of the highest genetic worth available (orchard or tested wild-stand seed) and to meet an acceptable level of genetic diversity.

[7] Scions, or cuttings, from a parent tree grafted onto established rootstock in the orchard.

[8] "Select" is used to describe seed and vegetative material exhibiting a level of genetic gain over wild-stand material. Thus, all seed and vegetative lots derived from orchards and production facilities (genetic Class A) and superior provenances (genetic Class B+) are now considered *select seed sources*. All seed and vegetative lots derived from natural stand sources not identified as superior provenances (i.e., genetic Class B) are now considered *standard seed sources*.

[9] A quantity of cones or seeds having uniformity of species, source, quality and year of collection.

4.0 Biotechnology Opportunities in Tree Improvement

Biotechnology is defined in the *Canadian Environmental Protection Act* as "the application of science and engineering in the direct or indirect use of living organisms, or parts of organisms, in their natural or modified forms." Biotechnology in plant science is receiving tremendous attention because of its potential changes to traditional tree breeding and seed production approaches.

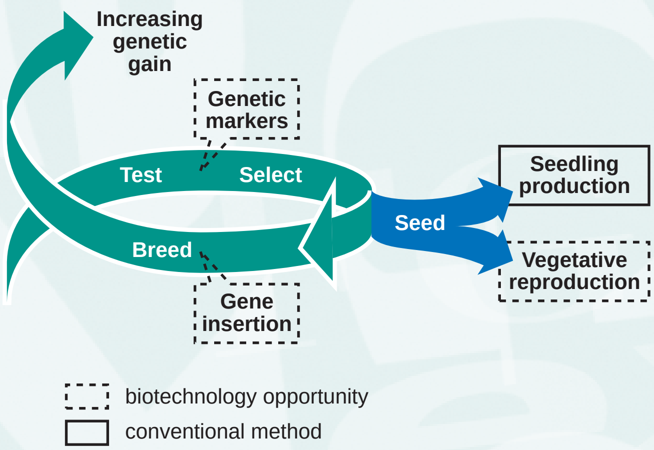

Figure 4
Biotechnology potential applications in tree improvement.

Potential applications of biotechnology in tree improvement fall into three main areas (Yanchuk 2001):

- new vegetative reproduction methods that aid in the production of improved planting stock
- genetic markers that can help researchers identify trees with desirable genes
- genetic engineering that can provide useful information about cell biology and function, and in the long term may help researchers produce trees with novel traits.

Figure 4 indicates opportunities for biotechnology to support tree breeding and seed production activities.

4.1 Vegetative Reproduction

Biotechnology has introduced new methods of vegetative reproduction—the growing of new plants from portions of existing plants. While trees have been vegetatively reproduced from rooted cuttings for ages, a new biotechnology—somatic embryogenesis (SE)—allows the production of embryonic plants from the somatic, or non-reproductive, tissue of a seed. The resulting "somatic seedlings" contain only the genetic material found in their parent trees, and in this way are similar to rooted cuttings. The primary difference between using cuttings and SE is the "unlimited" number of plants that theoretically can be produced from SE.

Although several trials are underway, SE is not used in British Columbia's reforestation programs. Other parts of the world, such as the southeastern United States and New Zealand, are beginning to move towards SE as one method of producing plants for reforestation in intensively managed, short-rotation tree farms. It will take several years of field testing and analysis to clarify the economic value and biological appropriateness of using SE in British Columbia.

4.2 Genetic Markers

Biotechnology has also introduced new ways to study genes and their functions through the use of genetic markers.

A genetic marker can be a whole gene, part of a gene, a sequence in non-coding DNA between genes, or an enzyme produced by a gene. The marker can be used to identify a location in a genome, or to identify an individual or group of related individuals. Different types of markers are used for different research applications.

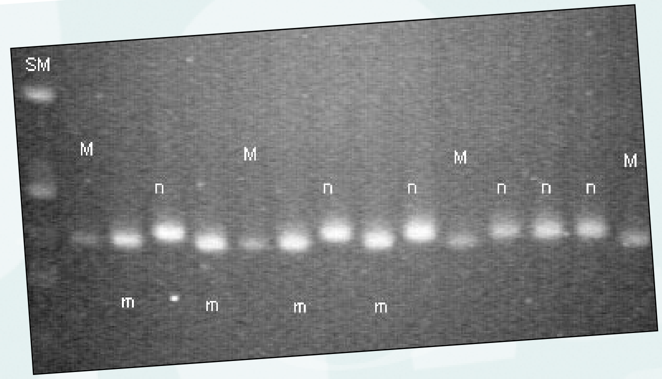

Figure 5
Agarose electrophoresis gel showing the result of a verification of control crosses. Chloroplast DNA marker of male parent "M" (four lanes) matches embryo chloroplast DNA "m" (four lanes). Six DNA samples from embryos "n" do not match the assumed male parent "M." SM indicates DNA size marker. Photo: M. Stoehr.

British Columbia forest researchers are working with genetic markers on several projects related to tree breeding. Studies using markers derived from chloroplast DNA are providing insights into the dynamics of pollen competition, the measurement of genetic worth, and the effectiveness of seed orchard management techniques.[10] Markers are also being used to help quantify levels of genetic diversity in natural and seed orchard populations of trees.[11]

In a seedling, the chloroplast DNA is inherited from the male parent, but can also be detected in the megagametophyte tissue of seeds to determine the female parent of seed in a bulked, wind-pollinated seedlot. Chloroplast DNA markers can, therefore, be used as molecular "fingerprints" to identify which seed came from which parent. To do this, the chloroplast DNA marker for each parent tree in a seed orchard must be identified, so that the chloroplast DNA for the seed or seedlot can be compared for matches with these known markers.

Figure 5 shows an example where ten embryo (seed) DNA bands (m, n) are compared to the band of an assumed male parent (M). The bands "m" and "M" match, establishing that M was the father. Embryo DNA bands "n" are at a slightly different location than M, which means that M was not the father.

Someday, genetic markers might make it possible to identify individual trees that carry genes for desired traits, and in this way help tree breeders to select trees more efficiently. This is not currently possible because of the complexity of traits such as growth, wood quality, and pest resistance, and our rudimentary understanding of the genetic processes controlling these traits.

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[10] See, for example, Stoehr, M.U. and C.H. Newton. [2001]; Stoehr et al (1999); Stoehr et al (1998).

[11] See, for example, Stoehr and El-Kassaby (1997).

4.3 Genetic Engineering

GE is the modification of an organism's genome by deliberately introducing genes or by removing or suppressing part of its genetic material.

Genetic engineering (GE) is the modification of an organism's genetic makeup, or genome, by deliberately introducing genes or by removing or suppressing a part of the organism's genetic material. Organisms that result from this process are currently referred to as "genetically modified organisms" (GMOs). While introduced genes may come from other individuals of the same species, they typically come from other species, such as viruses, bacteria, and other plants and animals, in which case they are referred to as "transgenic." The introduced genes may act only as genetic markers, or they may allow the organism to express a novel trait.

GE has the potential to produce novel traits that cannot be delivered through traditional tree breeding programs. Although it may be 15–20 years before a genetically engineered tree could be released for commercial use anywhere in Canada (A.-C. Bonfils, pers. comm., Jan. 2001), the use of GE technologies in tree genetics research may benefit tree breeding programs sooner.

Selective tree breeding and genetic engineering are fundamentally different processes.
Selective breeding combines genetic material from individuals of the same species through natural sexual reproduction. In contrast, GE modifies the genome, typically by inserting into a plant new genetic material—usually from a different species—on metal microprojectiles or on viral or bacterial vectors. In most cases, the new gene combinations produced through GE do not exist in nature and cannot be obtained through selective breeding.

Researchers at the Canadian Forest Service (CFS) and elsewhere are actively exploring the potential for using genetic markers to help identify trees with superior pest and disease tolerance, and to study genetic diversity in tree populations. They are also cautiously investigating the development and application of transgenic trees. The CFS Laurentian Forestry Centre in Quebec is currently conducting field trials of a transgenic spruce tree that resists insects by manufacturing the same toxins that are naturally produced by *Bacillus thuringiensis (Bt)*. *Bt* is a naturally occurring pathogen that is already widely used in spray form to manage forest pests.

Worldwide, forest researchers are looking at a range of other possible transgenic products, including trees with traits such as herbicide resistance, improved wood quality (modified amounts and types of lignin), faster growth, and sterility.

5.0 Is There a Future for Transgenic Trees in Canada?

Public opinion surveys by the federal government over the last few years indicate cautious public support for forest biotechnology, as long as questions about its potential impacts on biodiversity and the environment are addressed (A.-C. Bonfils, pers. comm., Jan. 2001).

Public values will have an important influence on the future of transgenic trees in Canada, because the Crown owns most forest lands.

One of the main concerns is the risk of transferring introduced genes from genetically engineered trees to wild populations through cross-pollination.[12] Another is whether transgenic trees will act in unpredictable ways, or will have qualities that enable them to out-compete their wild relatives (S. Aitken, pers. comm., Jan. 2001).

The introduction of trees with novel traits may affect ecosystem processes in subtle, undesirable ways. For example, built-in pesticides might be able to enter the food chain, affecting insect predators, scavengers, and decomposers. Or GE may alter the function of non-target genes in ways that are not readily apparent or detectable (Yanchuk 2001).

No such concerns exist with the planting stock used for reforestation in British Columbia today. Seedlings produced through conventional breeding contain no introduced genes—the genetic makeup of the planted trees is derived from the natural, well-adapted wild trees among which they are planted.

Given public concern, scientific uncertainty, and forests with high ecological, aesthetic, and commercial values, governments in Canada are understandably taking a cautious approach to transgenic trees. In British Columbia, the use of transgenic trees will only be allowed if it meets all federal and provincial ecological and biological safety regulations, and economic criteria that affect most reforestation and plantation investment schemes (A. Yanchuk, pers. comm., Feb. 2001). To date, the B.C. Ministry of Forests has not registered any transgenic seed or vegetative lots for operational reforestation on Crown land (D. Draper, pers. comm., Feb. 2001).

Over the next few years, governments in Canada will need to finalize a comprehensive national policy and regulations on transgenic trees. Discussions to this end are already underway. Experts from the federal and provincial governments, forest companies, universities, and other institutions have been meeting since 1996 to share information and to explore various issues related to forest biotechnology. British Columbia, as a major forest producer, is an important participant in these discussions.

[12] Such questions are particularly important in British Columbia, where almost three-quarters of the area harvested is regenerated with planted trees that could pollinate surrounding wild trees.

6.0 Conclusion

B.C. is undertaking research to improve understanding of where biotechnologies can augment traditional tree improvement activities, and the implications of their application.

Traditional tree breeding activities have substantially increased the quality of seedlings used for reforestation in British Columbia. By the end of this decade about 75% of the seedlings planted in this province will be grown from select seed produced through conventional tree breeding and seed orchard production.

Biotechnology may augment traditional tree improvement activities by providing valuable information to tree breeders and supplementing the production of high quality seed.

Genetic engineering may enable forest researchers to grow trees with new and desirable traits. Whether such knowledge will have widespread commercial application, however, remains uncertain. Significant technical challenges must be overcome before genetically engineered trees could be used for operational reforestation. The ethical issues of whether they should be used may be even more difficult to address.

At this point, British Columbia is focusing on research activities that will improve understanding of where biotechnologies can augment traditional tree improvement activities, and the implications of their application.

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